Scrapie in Immunologically Deficient Mice

ALTHOUGH the agent responsible for scrapie is classified as a slow virus, many features distinguish it from a typical virus[1-3], one of which is its apparent failure to elicit an immune response. Attempts to demonstrate antibody by neutralization[4,5], complement fixation[4], precipitation[4] and immunofluorescence[4,6] have been unsuccessful, but the role of cell-mediated immunity in scrapie has been less well studied.

Neonatal thymectomy did not alter the incubation period or pathology in one small series of mice inoculated intracerebrally with scrapie[2]. We have studied the course of scrapie produced by intraperitoneal inoculation in thymectomized mice, lethally irradiated and reconstituted with foetal liver cells. Although they were markedly depleted of thymus-derived (T) lymphocytes and were incapable of rejecting allogeneic skin grafts, their response to the scrapie agent was not different from that of normal mice.

CBA/He mice of both sexes were used: group I was thymectomized at 4 weeks old, irradiated with 900 r. and reconstituted with syngeneic foetal liver cells; group II received 900 r. and was reconstituted with foetal liver cells; group III was untreated. Four weeks later they all received skin grafts from A strain donors and after 9 days half the animals in each group were inoculated intraperitoneally with 0.25 ml. of 10^{-1} dilution of normal BSVS mouse brain; the rest received 0.25 ml. of 10^{-1} dilution of brain from BSVS mice with scrapie; these materials were supplied by Dr R. L. Chandler.

Cell-mediated immune function was followed by assessment of skin allograft survival. In addition the percentage of cells bearing the θ allo-antigen (a marker for T lymphocytes[7]) and the microscopic appearance of the lymphoid organs were studied in two animals from each group, 7 months after inoculation (Table 1). Animals in group I retained skin allografts throughout the experiment, had very few θ-bearing cells in their blood and were markedly depleted of lymphocytes in the thymus-dependent areas of spleen and lymph nodes[8]. In contrast, mice in groups II and III rejected their skin grafts after 13.6 and 10.3 days respectively, had normal numbers of θ-bearing blood lymphocytes and normal lymphoid organs.

Mice were observed five or six times a week for the appearance of scrapie following the criteria of Dickenson *et al.*[9]. No mice inoculated with normal brain developed signs of scrapie, but those injected with scrapie-affected brain developed scrapie and either were killed in extremis or died within 230 days of inoculation. Many of the immunologically deficient mice in group I died before developing signs of scrapie. None of these mice had neuropathological lesions consistent with scrapie during the clinical phase of the disease.

Table 2 shows that scrapie began between 163 and 170 days in all three groups. There was no significant difference between mean times of death of 190 and 205 days seen in groups I and III respectively; they were even closer than appears because four of the mice in group I were killed when judged to be preterminal in order to obtain satisfactorily histology and peripheral blood for determination of θ-bearing lymphocytes. Although the extent and magnitude of the nervous system lesions were not compared in detail, the neuropathological changes revealed by haematoxin and eosin or Cajal were consistent with scrapie in all three groups. Thus we could demonstrate no appreciable difference in the clinical and pathological aspects of scrapie in immunologically deficient mice depleted of T lymphocytes when compared with normal mice. This suggests that cell-mediated immunity neither contributes to the pathogenesis of scrapie nor plays a significant role in the host's defence against the agent.

Table 1 Cellular Immune Status

Group	Treatment	Skin allograft survival in days (mean)	% θ-bearing cells in blood*	Lymphoid* organs
I	Thymectomy 900 r. Reconstituted with foetal liver	Greater than 200	4, 17	Lymphocyte depletion in in thymus-dependent areas
II	900 r. Reconstituted with foetal liver	9–19 (13.6)	77, 68	Normal
III	None	9–14 (10.3)	74, 73	Normal

* Evaluated after 7 months.

Table 2 Time Course of Scrapie

Group	No. of mice	Onset of disease (days)	Time from inoculation to death (days) Range	Mean
I	7	163–170	174–200	190
II	16	163–170	200–223	215
III	24	163–170	173–228	205

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Extraction of Prostaglandins from Human Blood

EXISTING methods for extracting prostaglandins from tissues or seminal plasma with 70–90% ethanol[1] or acetone[2] give poor and inconsistent recoveries from blood. Equilibrium dialysis and recovery experiments with labelled and unlabelled prostaglandins show that this is due to binding with precipitated serum albumen (unpublished results of W. G. U.). Plasma globulins and intact blood cells do not seem to interact with the prostaglandins. When precipitation of protein is avoided by using 40–50% ethanol acidified with formic acid, the prostaglandins can be extracted quantitatively into chloroform. The method is as follows: (1) centrifuge heparinized or citrated blood; (2) mix 1 volume each of plasma and saline with 2 volumes of ethanol (analytical grade); (3) extract twice with petrol (boiling point 40°–60° C, 2 × 2 volumes) to remove neutral fats including carotene; (4) adjust to *p*H 3–3.5 with formic acid (1–3% v/v); (5) extract twice with amounts of chloroform each equal to the total volume in stage 4; (6) evaporate chloroform in a thin film evaporator at 30° C; (7) redissolve residue in 10 ml. chloroform and evaporate to aid the removal of formic acid; (8) blow oxygen-free nitrogen through